Stress and the Gastrointestinal Tract
III. Stress-related alterations of gut motor function: role of brain corticotropin-releasing factor receptors

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The influence of emotion on gastric motor function was first described as anecdotal clinical observations by Cabanis and Beaumont followed by Pavlov’s and Cannon’s pioneering experimental studies in cats and dogs at the beginning of the 1900s (see Ref. 32). Although Selye’s original concept of stress appeared as a short letter in Nature in 1936, in 1934 Hall established the use of defecation score as an index of emotionality in rats exposed to unfamiliar surroundings (9). Alterations of the gastrointestinal (GI) motility pattern and transit by various stressors were thereafter well documented with the development of quantitative techniques to monitor GI motility and transit in experimental animals and humans. However, it was only during the past two decades that attempts were made to unravel the mechanisms underlying the gut motor response to stressful stimuli. In particular, the characterization of the 41-amino acid peptide corticotropin-releasing factor (CRF) and a related family member, urocortin, the cloning of CRF receptor subtypes 1 (CRF-R1) and 2 (CRF-R2), and the development of specific CRF-R1 and CRF-R2 receptor antagonists, namely, α-helical CRF-(9–41), [Phe12,Nle38]h/rCRF-(12–41), and, more recently, astressin (cyclo(30–33)-[Phe12,Nle38,Glu39,Lys33]CRF-(12–41)) (15, 18) provided relevant novel tools to characterize the neurochemical basis of the stress response. Evidence has emerged that activation of CRF receptors in the brain mediates almost the entire repertoire of behavioral, neuroendocrine, autonomic, immunologic, and visceral responses characteristic of stress in rodents and primates (8, 11, 29). We review here the patterns of stress-related disturbances in GI motor function and recent advances made in the understanding of the central nervous system mechanisms underlying stress-induced alterations of gastric and colonic motor function, with particular emphasis on the role of brain CRF receptors.

Patterns of Gastrointestinal Motor Responses to Various Stressors

Acute stress induces differential motor effects in the upper and lower GI tract. Delayed gastric emptying is commonly induced by various acute stressors such as operant avoidance, water avoidance, radiation, handling, acoustic stimulation, hemorrhage, abdominal or cranial surgery, tail shock, trunk clamping, wrap restraint at room temperature, swimming, and anesthetic exposure in experimental animals (mice, rats, guinea pigs, dogs, and/or monkeys) (5, 24, 26). Likewise, in healthy human subjects, anger, fear, labyrinthetic stimulation, painful stimuli, preoperative anxiety, or intense exercise results in a slowing of gastric emptying (19, 24, 26). Acute exposure to wrap restraint, cold water swim, or ether also slows down...
intestinal transit in the rat (30). In contrast, colonic motility, transit, and/or defecation are stimulated by conditioned fear to inescapable foot shocks and exposure to water avoidance, tail shock, loud noise, open field test, and restraint at room temperature or in a cold environment, (5, 24). Similarly, in healthy human volunteers, painful stimuli, dichotomous listening test, fear, anxiety, anger or a stressful interview enhances colonic motor activity (19). Although the above gut motor changes represent the most characteristic patterns induced by various stressors, differential responses are also encountered after specific stimuli. In particular, acute exposure to cold, which is largely used to study gastric erosion formation induced by stress, activates both gastric and colonic motility and transit in rats (14, 24).

Gastrointestinal motor responsiveness to acute stress is affected by previous stress experiences and intrinsic biological factors such as the animal species, strain, gender, estrus cycles, circadian pattern, and regions of the GI tract. For instance, a short session of inescapable foot shocks induces long-term sensitization of the colonic contractile response to a novel stressor applied 2 wk later (21). Fischer rats are more sensitive than Sprague-Dawley rats to acute wray restraint-induced inhibition of small intestinal transit (30) and more sensitive than Lewis rats to water avoidance-induced defecation (16). Female rats are also more sensitive than males to the small intestinal inhibitory effect of chronic wray restraint (30). Circadian variations with a greater sensitivity to the effects of stress on intestinal transit were observed in the late afternoon in rats (30). With regard to regional differences, the colon is more responsive to stress than the upper gut (5, 22). However, reports on the modulation of the gut motor response to acute stress by these factors are relatively scarce, and underlying mechanisms remain poorly characterized. Another important limitation of studies on stress-related alterations of GI motility is their focus on short-term stress while the consequences of long-term exposure to homotypic or heterotypic stressors have received little attention (31).

**BRAIN CRF RECEPTORS AND STRESS-RELATED INHIBITION OF GASTRIC MOTOR FUNCTION**

Several reports consistently established that CRF injected into the cerebrospinal fluid (CSF) acts in the brain to inhibit gastric emptying of a solid or liquid meal and contractility in rats and dogs (25, 26). The paraventricular nucleus of the hypothalamus (PVN) is a responsive site for CRF-induced delayed gastric emptying (26). In addition, CRF in the dorsal vagal complex (DVC) inhibits central vagal stimulation of gastric motility in rats (26). The central action of CRF is receptor mediated, as shown by the use of CRF antagonists, of which the recently developed astressin is 16- and 6-fold more potent than α-helical CRF-(9–41) and [α-Phe12,Nle21,38]h/rCRF-(12–41), respectively (26). Indirect pharmacological evidence suggests that intracranial CRF-induced inhibition of gastric emptying involves interaction with CRF-R2 in rats (13, 25).

The CRF receptor antagonists α-helical CRF-(9–41), [α-Phe12,Nle21,38]h/rCRF-(12–41), and astressin, injected into the CSF or the PVN at doses preventing gastric response to centrally injected CRF, block the delayed gastric emptying induced by various forms of stress, immunologic (intravenous or intracisternal injection of interleukin-1β), physiopsychological (partial restraint, forced swimming), chemical (ether), or visceral (abdominal or cranial surgery or peritoneal irrigation induced by intraperitoneal injection of acetic acid) (Ref. 26; Fig. 1). Astressin is more potent and efficacious than previously developed CRF antagonists (26). In contrast, peripheral administration of these CRF antagonists has no effect on the gastric response to stress except for abdominal surgery (26). These findings opened new venues for understanding brain and peripheral pathways contributing to postoperative ileus (2). It also revealed that the intercommunications between the immune and hypothalamic CRF systems impact on the central regulation of gastric motor function. The relationship between the immune and neuroendocrine systems has been well defined with respect to mechanisms and neuronal circuitries through which immune signals activate the hypothalamic-pituitary-adrenal axis (20). However, central CRF-induced delayed gastric emptying is independent from the activation of pituitary-adrenal hormone release. It is related to the alterations of autonomic nervous system activity, in which the decrease in gastric vagal outflow plays a role (26). The cellular mechanisms through which cytokines of peripheral or central origin activate brain CRF pathways involved in the autonomic regulation of gastric motor function require elucidation.

Different stress paradigms, in addition to CRF, produce diverse neurochemical alterations and dissimilar changes in circulating hormone levels (10). For instance, cold exposure in rodents illustrates a stressorspecific activation of thyrotropin-releasing hormone (TRH)-containing neurons in the PVN and medulla designed to coordinate thermoregulatory adaptive processes through stimulation of the autonomic nervous system (1, 33). Of relevance to GI function, TRH exerts a direct postsynaptic excitatory effect on dorsal motor nucleus (DMN) neurons through interaction with TRH receptors, leading to increased gastric vagal efferent discharges (33). Activation of medullary TRH receptors mediates the stimulation of gastric contractility and emptying induced by acute exposure to cold (14). The cold-specific activation of medullary TRH neurons (33) and related vagal activation to the gut provide insight to the differential gastric motor response to cold (stimulatory) compared with other stressors (inhibitory) (14). However, central CRF inhibits DMN neurons activated by TRH (26). Such interplay may modulate the outcome of gastric response to sustained cold as indicated by the dampening of gastric contractile response under these conditions.
BRAIN CRF RECEPTORS IN STRESS-RELATED STIMULATION OF COLONIC MOTOR FUNCTION

CRF injected into the CSF increases colonic motility, decreases colonic transit time, and induces fecal excretion, reproducing colonic motor response to various stressors. CRF brain sites of action characterized so far are the PVN and locus ceruleus complex (26). Transneuronal retrograde tracing studies with pseudorabies virus inoculated into the distal colon provide neuroanatomic support that these sites are synaptically connected to regulate colonic function through bulbospinal pathways (28). An interesting novel report showed that CRF projections from the Barrington nucleus innervate sacral parasympathetic preganglionic neurons that are synaptically linked to the distal colon (27). This suggests another potential sacral spinal site of action for CRF to influence colonic motility.

The role of CRF receptors in mediating stress-related activation of colonic motor function is supported by the demonstration that α-helical CRF-(9–41), [D-Phe², Nle¹⁷]h/rCRF-(12–41), and more potently, astressin injected into the lateral ventricle, PVN, or locus ceruleus complex abolished partial wrap restraint- or water avoidance-induced stimulation of colonic transit and defecation as well as conditioned fear-induced increase in cecal and colonic spike burst frequency (16, 26) (Fig. 1). Likewise, interleukin-1β and neuropeptides such as glucagon-like peptide 1 and neuropeptide Y, all known to act in the brain to activate hypothalamic CRF-containing neurons when administered into the brain, stimulate colonic motor function as assessed by decrease in colonic transit time, increase in defecation, or enhanced spike burst frequency, through central CRF receptor-dependent mechanisms (7, 17, 26). Conversely, Lewis rats with a defective hypothalamic CRF response to immune challenges and other stressors exhibit a 50% reduction in pellet output induced by water avoidance stress that is associated with a decrease in neuronal activation in the PVN and sacral parasympathetic nucleus (16).

Pharmacological characterization of CRF receptor subtype using selective nonpeptide CRF-R1 antagonists, namely CP-154526 and NBI-27914, indicates that the CRF-R1 subtype is primarily involved in the stimulation of colonic propulsion induced by central administration of CRF and one stressor tested so far, water avoidance in rats (12, 13). Of significant relevance is that the anxiogenic behavioral response to
central administration of CRF or stress is also mediated by brain CRF-R1. This was established by the use of selective CRF-R1 antagonists, CRF-R1 antisense, and CRF-R1 knockouts (23). In addition, there is an overlap between hypothalamic and pontine nuclei responsive to CRF that mediate the anxiogenic behavior and stimulation of colonic motor function through CRF-R1 (11, 13, 26). Brain circuitries that activate anxiogenic and colonic motor function under stress through CRF-R1-dependent pathways may provide the neurobiological basis for the observed relationship between emotionality and defecation in rats. Such mechanisms may also have clinical relevance in the context of established association between anxiety/depression and irritable bowel syndrome (IBS) (3). Recent findings in monkeys and humans indicate that the CRF-R1 antagonists antalarmin and R-121919 alleviate manifestations of anxiety and depression (8, 34). This preliminary report of beneficial effects of CRF-R1 antagonist treatment on depressive symptoms in patients with major depression (34) suggests a therapeutic potential for CRF-R1 antagonists, particularly in subsets of IBS patients who have psychiatric illness and GI symptoms of enhanced bowel motor function.

CONCLUSIONS

There is overwhelming experimental and clinical evidence that stress influences GI motility. The most consistent pattern of GI motor alterations induced by various acute stressors is that of delaying gastric emptying while accelerating large bowel transit, which is mimicked by central administration of CRF. Several convergent findings implicate the activation of brain CRF receptors in mediating the delayed gastric emptying and stimulation of colonic motor function resulting not only from acute exposure to psychological, physical, or chemical stress but also from activation of the immune system (Fig. 1). Novel findings related to the differential CRF receptor subtypes involved in the central actions of CRF on upper and lower gut transit point to a role of medullary CRF-R2 in the inhibition of gastric emptying. In contrast, central injection of CRF and psychological stress act through CRF-R1 to induce anxiogenic and colonic motor responses. The exact neuronal circuitries whereby CRF interacts with CRF-R1 and CRF-R2 receptors and how it translates into autonomic-dependent alterations of GI motor function in response to various acute stressors must be further elucidated. Because most of the knowledge on stress-related GI motor alterations is derived from acute exposure, the impact of chronic homotypic or heterotypic stressors must be characterized. Whether CRF-R1 in the PVN that are upregulated during chronic restraint (4) may have a bearing in the colonic sensitization after stressful experience (21) is worth exploring. Likewise, the influence of early postnatal stressful life events that alter the central CRF response to stressors in adulthood (6) will be a relevant model to explore vulnerability to stress-related GI motor alterations. The first clinical report that CRF-R1 antagonist reduces depression and anxiety scores in depressed patients and the experimental evidence for a role of CRF-R1 in mediating stress-related anxiogenic behavior and stimulation of colonic motor function in rodents (12, 13) suggest a possible therapeutic value of CRF-R1 antagonists in the context of IBS patients with depression/anxiety or chronic stress.

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